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Commentary

Re-probing DNA Blots: Wet is Better than Dry Storage of Uncharged Nylon Membranes After Removing Probes

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Abstract. DNA immobilized on a nylon membrane can be re-probed multiple times with different probes. Protocols typically recommend that DNA blots be stored either dry at room temperature or wet at 4 or –20°C after a probe is removed. This study shows substantial differences in the effect of these storage options on the performance of uncharged nylon membranes in subsequent hybridizations. Uncharged membranes, air-dried and stored at room temperature after probe removal, could not be successfully re-probed. However, excellent rehybridization results were obtained following probe removal when wet membranes were wrapped in plastic and stored at –20°C.

Key words: DNA detection, probe hybridization, RFLP analysis, stripping Southern blots

Abbreviations: BSA, bovine serum albumen; CTAB, hexadecyltrimethylammonium bromide; EDTA, disodium ethylenediamine tetraacetate; $NaHPO_4$, sodium phosphate; SDS, sodium dodceyl; SSC, sodium citrate.

Introduction

A common technique used in plant research DNA analysis is hybridization of sequence specific probes to DNA immobilized on a nylon membrane. One advantage of transferring DNA to a nylon membrane is that it can be re-probed multiple times using different probes. Re-probing typically entails removing ("stripping") the probe with either heated or basic solutions prior to rehybridization with a different probe (Davis et al., 1994; Anderson, 1999).

After probe stripping, published protocols recommend either that membranes be air dried and stored at room temperature (Tijssen, 1993; Ausubel et al., 1994) or that they be wrapped in plastic while damp and placed at 4°C (Davis et al., 1994) until the next use for hybridization. Sometimes authors indicate that both storage options are acceptable (Anderson, 1999), suggesting that little difference may be expected in the performance of a membrane in subsequent

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hybridizations. Dry, room temperature storage of membranes after stripping would seem logical since this is the storage method recommended prior to initial probing following DNA fixation (Sambrook et al., 1989). However, Giusti et al. (1992) found that different storage conditions resulted in significant differences in rehybridization following probe removal with two different types of positively charged membranes. They found variable results for rehybridization of membranes that had been air-dried and stored at ambient conditions. Alternatively, rehybridization was always successful if the membranes were wrapped in plastic while wet and stored at 4 or –20°C.

Our laboratory examines genetic variability in plant species by repeated hybridizations, using different multilocus variable-number-of-tandem-repeat (VNTR) probes, to endonuclease-digested genomic DNA immobilized on uncharged nylon membranes (Rogstad, 1996; Keane et al. 1999). Stripping procedures often cause signal reduction when subsequent probes are hybridized to the membrane. In our experience, this signal reduction is frequently quite severe with membranes that were dried and stored at room temperature. In fact, following the successful initial probing of a membrane all subsequent probings after stripping often fail to yield any signal. These observations and the findings of Giusti et al. (1992) led us to perform experiments to determine whether dry, room temperature storage of probed and stripped uncharged nylon membranes can result in severe signal reduction upon re-probing.

Materials and Methods

Membrane preparation

Genomic DNA was obtained from Ohio buckeye (*Aesculus glabra*) and dandelion (*Taraxacum officinale*) plants by grinding 1 g of leaf tissue in liquid nitrogen and incubating the powdered tissue for 24 h at 68°C in 12 ml 2× CTAB with 1% 2-mercaptoethanol. Following incubation, DNA was extracted and gel purified as described in Rogstad (1993). Genomic DNA was digested with a fivefold excess of the endonuclease *Hae* III (buckeye) or *Taq* I (dandelion) according to the manufacturer's instructions (New England Biolabs). The resulting DNA fragments were size separated by electrophoresis (2-3 µg DNA/lane) in submerged 1.2% agarose gels and transferred to nylon membranes (either Biodyne A, Pall; MagnaGraph, Micron Separations Inc.; or Duralon-UV, Stratagene) by Southern blotting in 10× SSC. DNA on all filters was immobilized by first UV crosslinking (Stratagene Stratalinker 2400 per instructions) and then baking for 2 h at 67°C. After baking, each membrane was stored between sheets of Whatman 3MM paper in a sealed plastic bag at room temperature for 2-6 d prior to initial hybridization.

Membrane hybridization and stripping

The blotted nylon membranes were hybridized with radiolabeled, polymerase chain reaction-synthetic tandem repeat probes produced according to Rogstad (1993). The core sequences of the two probes used in these hybridizations were GATA and TTCCA. Membrane prehybridization and hybridization were both conducted overnight at 60°C in a 0.263 M $NaHPO_4$, 7% SDS, 1 mM EDTA and

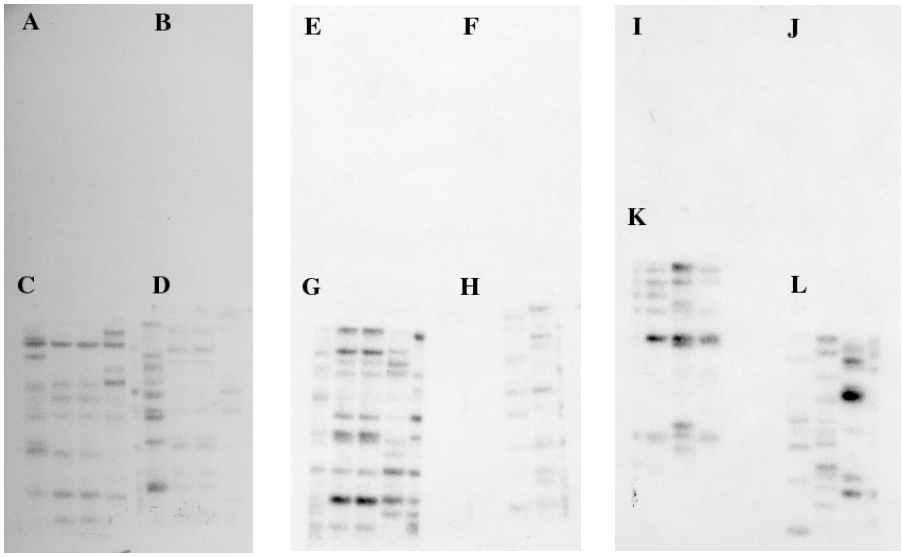

Figure 1. Autoradiographs of uncharged nylon membranes probed with the GATA repeat probe. Blots contain *Hae* III-digested DNA from different Ohio buckeye (*A. glabra*) individuals. Membranes A-D are Biodyne A, E-H are MagnaGraph, and I-L are Duralon-UV. Only membranes A, B, E, F, I, and J were stripped, air dried and stored 24 h at room temperature prior to probing. Membranes C, D, G, H, K, and L were not stripped prior to this probing.

0.65% BSA solution. All membranes were washed twice for 1 h at 60°C with 1.8-2× SSC, followed by a 5 min rinse in 1× SSC at room temperature. Membranes were then placed wet in plastic wrap for autoradiography at –70°C (Kodak Biomax MS film with intensifying screens). Following autoradiography, the wet membranes were stripped for 3 min in a 95°C solution of 0.1% SDS and rinsed twice in distilled water. Membranes were then either placed wet in plastic wrap and stored at –20°C, or air dried and stored at room temperature between sheets of Whatman 3MM paper in a sealed plastic bag until re-probing.

Results and Discussion

To test the hypothesis that dry membrane storage results in reduced hybridization signals, three sets of four DNA blots were prepared using Biodyne A, MagnaGraph, and Duralon-UV membranes. For each membrane type, all blots were made from the same membrane sheet and blotted from the same gel. Blots were Southern transfers of restriction enzyme digested DNA from different Ohio buckeye plants electrophoresed in different lanes. Half of the membranes of each type were then subjected to the probe stripping procedure and stored dry at room temperature for one day before hybridization. Simultaneous hybridization of all 12 membranes with the GATA repeat probe revealed signals only with control membranes that had not been stripped and air dried prior to being hybridized (Figure 1).

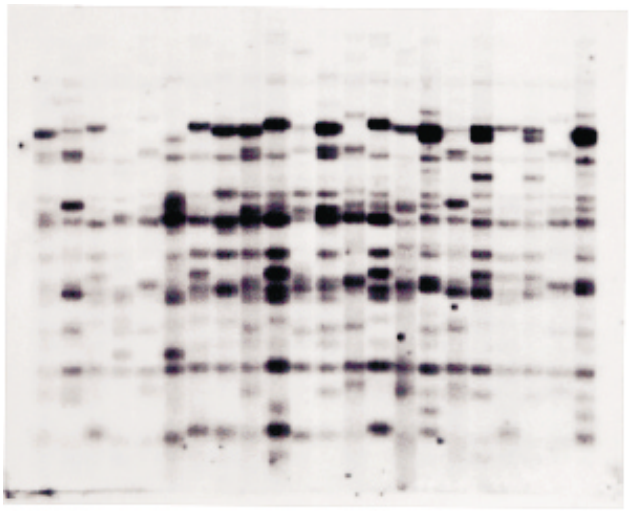

Figure 2. Autoradiograph of *Taq* I-digested DNA from 22 dandelion plants (*T. officinale*) probed with the TTCCA repeat probe. The membrane was initially probed in 1993, stripped, and then stored wet in plastic wrap at –20°C until this re-probing in 1999.

Thus, although published protocols may recommend that membranes be dried and stored at room temperature following stripping (Tijssen, 1993; Ausubel et al., 1994; Anderson, 1999), this method is, at best, variable in our laboratory (data not shown) and is contraindicated by the results presented here. Based on these results and those of Giusti et al. (1992), we now store probe-stripped DNA blots wet in plastic wrap at –20°C until re-probing. With Southern blots on membranes stored this way, excellent results (i.e., little reduction in signal intensity) have been obtained for rehybridization with up to 10 different probes. Indeed, membranes that were initially probed in June, 1993, stripped, and stored wet in plastic wrap at –20°C were successfully re-probed in March, 1999 (Figure 2). Thus, storing nylon membranes wet at –20°C following stripping seems to have eliminated the severe signal reduction often encountered after dry membrane storage.

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